

Q11 base sequence of about 4 kb Hind IIIHind III fragment was determined (Sequence Listing
SEQ ID NO:1.

Page 48, after the last line beginning on a new page, please replace the original
Sequence Listing with the substitute Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as shown on the marked-up copy following this amendment
to read as follows.

Q13 6. (Amended) A DNA encoding the sorbitol dehydrogenase as claimed in claim 1.

Q14 8. (Amended) The DNA of claim 6, which is derived from bacteria belonging to the
genus *Gluconobacter*.

Q15 12. (Amended) A recombinant vector comprising a DNA as claimed in claim 6.

13. (Amended) An expression vector comprising a DNA as claimed in claim 6.

Q16 15. (Amended) A transformant obtained by transforming a host cell with an
expression vector of claim 13.

17. (Amended) The transformant of claim 15, which is capable of converting D-
sorbitol to 2-keto-L-gluconic acid.

Q17 18. (Amended) A method for producing a protein having a sorbitol dehydrogenase
activity, which method comprises culturing a host cell transformed with an expression vector
of claim 13 in a medium and harvesting the sorbitol dehydrogenase of claim 1 or the protein
of claim 10 from the obtained culture.

Q18 22. (Amended) A method for producing L-ascorbic acid or an alkali metal salt
thereof or an alkaline earth metal salt thereof, which method comprises converting 2-keto-L-

Q18 gluconic acid obtained by the method of claim 20 to L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof.

Please add new Claims 23-48.

23. (New) A DNA encoding the sorbitol dehydrogenase as claimed in claim 5.

24. (New) The DNA of claim 23, which is (a) or (b) in the following:

(a) a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:1

(b) a DNA capable of hybridizing to the base sequence of the above-mentioned (a) under stringent conditions.

25. (New) The DNA of claim 23, which is derived from bacteria belonging to the genus *Gluconobacter*.

Q19 26. (New) A recombinant vector comprising a DNA as claimed in claim 23.

27. (New) A recombinant vector comprising a DNA as claimed in claim 9.

28. (New) An expression vector comprising a DNA as claimed in claim 23.

29. (New) An expression vector comprising a DNA as claimed in claim 9.

30. (New) The expression vector of claim 28 further comprising a DNA encoding sorbose dehydrogenase and/or a DNA encoding sorbosone dehydrogenase.

31. (New) The expression vector of claim 29 further comprising a DNA encoding sorbose dehydrogenase and/or a DNA encoding sorbosone dehydrogenase.

32. (New) A transformant obtained by transforming a host cell with an expression vector of claim 28.

33. (New) A transformant obtained by transforming a host cell with an expression vector of claim 29.

34. (New) The transformant of claim 32, which belongs to a genus selected from the group consisting of *Escherichia coli*, the genus *Pseudomonas*, the genus *Gluconobacter*, the genus *Acetobacter* and the genus *Pseudogluconobacter*.

35. (New) The transformant of claim 33, which belongs to a genus selected from the group consisting of *Escherichia coli*, the genus *Pseudomonas*, the genus *Gluconobacter*, the genus *Acetobacter* and the genus *Pseudogluconobacter*.

36. (New) The transformant of claim 32, which is capable of converting D-sorbitol to 2-keto-L-gluconic acid.

37. (New) The transformant of claim 33, which is capable of converting D-sorbitol to 2-keto-L-gluconic acid.

219 38. (New) A method for producing a protein having a sorbitol dehydrogenase activity, which method comprises culturing a host cell transformed with an expression vector of claim 13 in a medium harvesting the sorbitol dehydrogenase having the following properties

(a) action: catalyzes the reaction converting D-sorbitol to L-sorbose

(b) molecular weight: about 54 kDa

(c) coenzyme: NAD(P)⁺ dependent

(d) substrate specificity: specifically oxidizes sorbitol, mannitol and arbutol, but does not act on xylitol, ribitol, inositol or glycerol, or a protein derived from the genus *Gluconobacter*, which is encoded by a gene encoding a protein having a sorbitol dehydrogenase activity, which is a DNA capable of hybridizing a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:1 and a partial DNA thereof, and which has sorbitol dehydrogenase activity, from the obtained culture.

39. (New) A method for producing a protein having a sorbitol dehydrogenase activity, which method comprises culturing a host cell transformed with an expression vector of claim 28 in a medium and harvesting the sorbitol dehydrogenase having the following properties

(a) action: catalyzes the reaction converting D-sorbitol to L-sorbose

(b) molecular weight: about 54 kDa

(c) coenzyme: NAD(P)⁺ dependent

(d) substrate specificity: specifically oxidizes sorbitol, mannitol and arbutol, but does not act on xylitol, ribitol, inositol or glycerol, or a protein derived from the genus *Gluconobacter*, which is encoded by a gene encoding a protein having a sorbitol dehydrogenase activity, which is a DNA capable of hybridizing a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:2 and a partial DNA thereof, and which has sorbitol dehydrogenase activity, from the obtained culture.

40. (New) A method for producing a protein having a sorbitol dehydrogenase activity, which method comprises culturing a host cell transformed with an expression vector of claim 29 in a medium and harvesting the sorbitol dehydrogenase having the following properties

(a) action: catalyzes the reaction converting D-sorbitol to L-sorbose

(b) molecular weight: about 54 kDa

(c) coenzyme: NAD(P)⁺ dependent

(d) substrate specificity: specifically oxidizes sorbitol, mannitol and arbutol, but does not act on xylitol, ribitol, inositol or glycerol, or

a protein derived from the genus *Gluconobacter*, which is encoded by a gene encoding a protein having a sorbitol dehydrogenase activity, which is a DNA capable of hybridizing a DNA having a base sequence of base numbers 537 - 1991 of the base sequence depicted in Sequence Listing SEQ ID NO:1 and a partial DNA thereof, and which has sorbitol dehydrogenase activity, from the obtained culture.

41. (New) A method for producing an L-sorbose, which method comprises culturing a host cell transformed with an expression vector of claim 28 in a medium and bringing D-sorbitol into contact with the obtained culture or a treated product thereof.

42. (New) A method for producing an L-sorbose, which method comprises culturing a host cell transformed with an expression vector of claim 29 in a medium and bringing D-sorbitol into contact with the obtained culture or a treated product thereof.

219 43. (New) A method for producing 2-keto-L-gluconic acid, which method comprises culturing a host cell transformed with an expression vector containing a DNA encoding sorbose dehydrogenase and a DNA encoding corbosone dehydrogenase in a medium and bringing the L-sorbose obtained according to the method of claim 41 into contact with the obtained culture or a treated product thereof.

44. (New) A method for producing 2-keto-L-gluconic acid, which method comprises culturing a host cell transformed with an expression vector containing a DNA encoding sorbose dehydrogenase and a DNA encoding corbosone dehydrogenase in a medium and bringing the L-sorbose obtained according to the method of claim 42 into contact with the obtained culture or a treated product thereof.

45. (New) A method for producing 2-keto-L-gluconic acid, which method comprises culturing the transformant of claim 36 in a medium and bringing D-sorbitol into contact with the obtained culture or a treated product thereof.

46. (New) A method for producing 2-keto-L-gluconic acid, which method comprises culturing the transformant of claim 32 in a medium and bringing D-sorbitol into contact with the obtained culture or a treated product thereof.

219 47. (New) A method for producing L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof, which method comprises converting 2-keto-L-gluconic acid obtained by the method of claim 43 to L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof.

48. (New) A method for producing L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof, which method comprises converting 2-keto-L-gluconic acid obtained by the method of claim 44 to L-ascorbic acid or an alkali metal salt thereof or an alkaline earth metal salt thereof.
